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COUPLED RINGS IN RNA NANOTUBES AND PROPERTIES OF BIOLOGICAL NANOCLUSTERS

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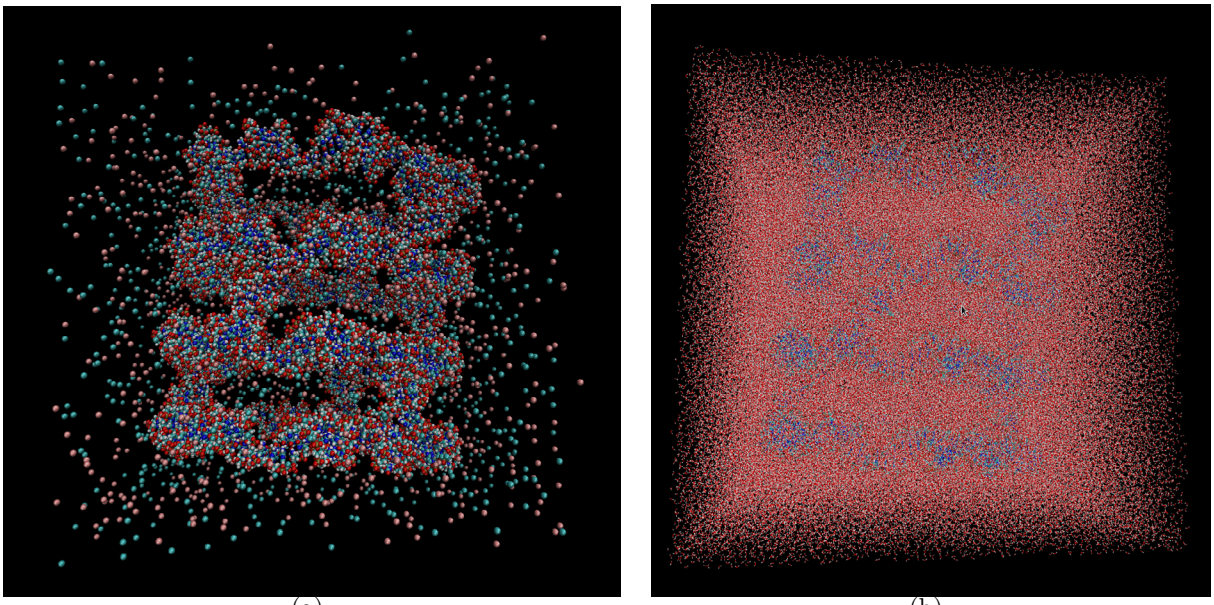
Abstract. In this contribution, we study biological nanoclusters where the coupling between their parts is essential. The main focus is on the RNA-based nanostructures. RNA molecules are very flexible in nature. This feature allows us to build various motifs which are indispensable in bio- nanotechnological applications. Based on the previous studies on RNA nanoclusters, in this contribution we analyze the structure and properties of RNA nanotubes, where we focus on nanotubes consisting of a series of coupled nanorings of around 20nm in diameter. We did a molecular dynamics (MD) simulation using CHARMM force field implemented in the NAMD package to study the structural and thermal properties of the nanotube in physiological solutions. Specifically, we have studied the behavior of these nanoclusters in solutions of NaCl, MgCl2 and KCl. We have analyzed such characteristics as the Root Mean Square Deviation (RMSD), the radius of gyration, the number of hydrogen bonds per base pairs, and the ionic distribution around the surface of the RNA nanocluster under study in different solutions. The variation of energy and temperature with simulation time have also been studied for all sets of simulations. The change in these features with the nature of solution has also been studied.

1 Introduction

Unlike most of the other biological molecular systems, RNA is structurally more flexible in nature which makes it an important biomolecular system. One of the most important application of the RNA nanotechnology research is in the field of biomedicine and bioengineering [1, 2]. For these applications it is very important to built the proper self assembly of the RNA building blocks. In our earlier studies, the modeling of RNA nanoclusters has been done by using the RNAI/II building blocks [3, 4, 5, 6]. The RNAI/II building blocks are the RNA strands which are taken from the protein data bank with the pdb code (2bj2.pdb) [7]. The elements of these RNA strands are the nucleobases Cytosine (C) , Guanine (G), Adenine (A), Thymine (T) and Uracil (U).



(a) (b)

Figure 1: VMD generated image of the RNA nanotube immersed into the MgCl2 Solution (a) Water molecules not displayed (b) With water molecules displayed.

The building blocks of these RNA nanoclusters i.e the RNAI and RNAII are defined as the sense and anti sense plasmids that control the replication of COLE1 [8, 9]. COLE1 is a DNA molecule separated from chromosomal DNA that is found in the cell of bacteria. The sequences for the RNAI is (GGCAACGGAUGGUUCGUUGCC) and that for the RNAII is (GCACCGAACCAUCCGGUGC) [7]. The six helical segments are constructed from RNAI and RNAII building blocks to model the RNA nanoring and the nanorings are connected via the links which are also built from the RNA strands of the size 22 nucleotides. The model structure of the RNA nanocluster is presented in Figure 1.

Experimentally the studies have been done for the calculation of free energy of RNA hairpin folding at different concentration of NaCl and KCl solutions [10] and found that the value of the free energy is depending on the logarithmic of the salt concentration as well as the stability of the RNA hairpin in NaCl solution was found more in comparison to the stability in in the KCl solution . The ionic concentration dependence studies on the binding of HIV -B virus has also been studied and it has been found that the binding of two human immunodeficiency viruses type-1 (HIV-1) is much more favourable at high concentration of salt in the presence of magnesium ion [11, 12]. Furthermore, the dependence of the self assembly of the tecto RNA has also attracted interest [11]. Recently, the several salt solutions has been used to study the detection of the MicroRNAs taking the DNA/silver nanocluster as the probe [13]. Having this in mind, in our current study the calculation of properties of RNA nanoclusters in different kind of solutions are performed for various type of ions.

The rest of this paper is organized as follows. In section 2 we describe the computational

methodologies used for calculations. The results are presented and discussed in Section 3. Concluding remarks and an outlook are then stated in Section 4.

2 Computational Details

In the modern development of the computational methods, the classical Molecular Dynamics simulation is one of the most versatile tools for the modeling of the biological systems [14, 15]. Here in our present contribution we have solvated the RNA nanocluster in a water box. To this solvated RNA nanocluster, we have added the K^+, $^{25}Mg^{2+}$and the $^{35}Cl^-$ions in order to make the RNA nanotube immersed in to the varieties of the salt solutions which can facilitate us to see the solution dependent behaviour of the RNA nanocluster The solvation and the ionization of the RNA nanocluster is done by using the VMD tool [16]. In order to do the molecular dynamics simulation of this RNA nanocluster at the constant temperature we have used the CHARMM27 force field [17] implemented by the software package NAMD [18].

In molecular dynamics simulation the classical equations of motion of a molecular system are solved by their time dependent integration. The potential of the system used during the molecular dynamics simulation using CHARMM force filed can be expressed as follows [17]:

$$V_{total} = \sum_{bond} K_b(r-r_0)^2 + \sum_{angle} K_\theta(\theta-\theta_0)^2 + \sum_{dihedral} K_\phi(1+cos(n\phi-\gamma)) + \sum_{Hbond}(\frac{C_{ij}}{r_{ij}^{12}} - \frac{D_{ij}}{r_{ij}^{10}}) + \sum_{Vanderwaals}(\frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^{10}}) + \sum \frac{q_{ij}}{\varepsilon r_{ij}} \quad (1)$$

where the first term corresponds to bonds, second corresponding to angle parameters and so on as indicated in the Equation 1 defining the potential of the system.

The output of the molecular dynamics simulation has also been analysed by using VMD to calculate the radial distribution, ion distribution, RMSD and the radius of gyration at different concentrations of salt solution. The RNA-nanotube modeled from the RNA building blocks has been solvated in a water box. The size of the box is taken in such a way that the distance from the surface of nanocluster to the wall is slightly larger than the cut off radius used in the MD simulation. In order to make the system neutral we have added 1254 $^{23}Na^+$ to the nanotubes. On top of this, before the MD simulation the RNA nanotube with four nanorings is ionized with the 1254 $^{23}Na^+$ and $^{35}Cl^-$ ions added to the waterbox. In the case of the MgCl2 solution we have added 1254 $^{25}Mg^{2+}$and $^{35}Cl^-$ions to the solvated RNA nanocluster system prepared for the molecular dynamics simulation. In case of the MgCl2 solution the $^{25}Mg^{2+}$ions being doubly positive charged, the half of this will contribute to neutralization of the phosphate group's negative charges in the entire tube and the rest of this will contribute to the added $^{35}Cl^-$ions. These systems were first simulated at constant temperature and pressure using NAMD software. The temperature in the system has been controlled by using Langevin's method with damping parameter $\eta = 5$ ps^{-1}. For adding chemical bonds between the segments in the nanoclusters we have used the topotools available in the VMD.

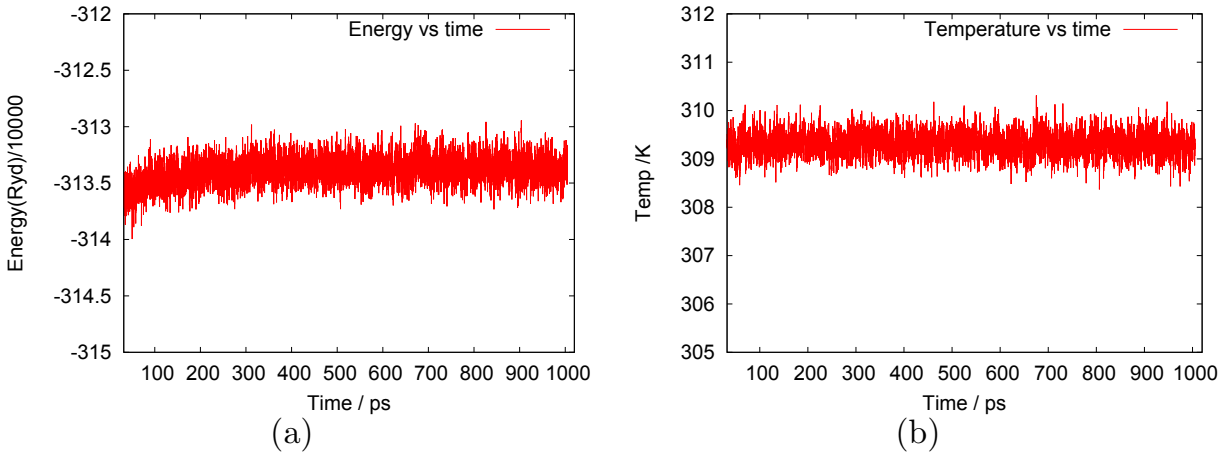


Figure 2: (a) Energy vs time and (b) Temperature vs time during the molecular dynamics simulation of 4 ring RNA nanotube in magnesium chloride solution.

3 Results and Discussion

In this contribution we focus on the behavior of RNA nanoclusters in the varieties of the metal chloride solutions including the physiological solution. The RNA nanoclusters are built from RNAI/II building blocks using molecular dynamics simulation. The construction of the RNA nanoclusters using these RNA building blocks have been provided in detail in our earlier work [4, 5]. The molecular dynamics simulations of the RNA nanotube have been performed in the simulation box for the time period 1ns at a constant temperature 310K. The energy and temperatures are saved in every 1ps. Variation of energy and the temperature during molecular dynamics simulation for the RNA nanotube with four RNA nanorings in the magnesium chloride solution is shown in Figure 2. From the energy plot it is clear that the energy at the beginning of the simulation goes on increasing but after about the simulation time 0.2ns the energy of the system becomes stable. The significant change in the energy is observed in the minimization region of the molecular dynamics simulation. The later part of the simulation is known as the production region around which we will do the calculations of the properties of the RNA nanocluster in the MgCl2 and NaCl solutions.

The number of $^{23}Na^{+}$, $^{25}Mg^{2+}$and $^{35}Cl^{-}$ions around the surface of the RNA nanotube within the distance 5 $\AA$ from its surface as a function of time in NaCl and MgCl2 solutions are presented in the Figure 3 (a). The trajectory of the molecular dynamics simulation is saved for 1ns time range in each of the solutions at 310K temperature. From these ionic distribution plots we see that the number of metal ions (i.e $^{23}Na^{+}$and $^{25}Mg^{2+}$) around the RNA nanotube surface are significantly higher than the number of $^{35}Cl^{-}$ions in both the solutions. The number of $^{25}Mg^{2+}$ions is less then the number of $^{23}Na^{+}$ions because the former is the divalent positive charged ion whereas the later is the monovalent singly charged ion. For a particular type of ions the number of ions at the beginning increases and approximately after 0.4ns the number of ions becomes consistent. The distribution of the $^{25}Mg^{2+}$and $^{35}Cl^{-}$ions around the surface of

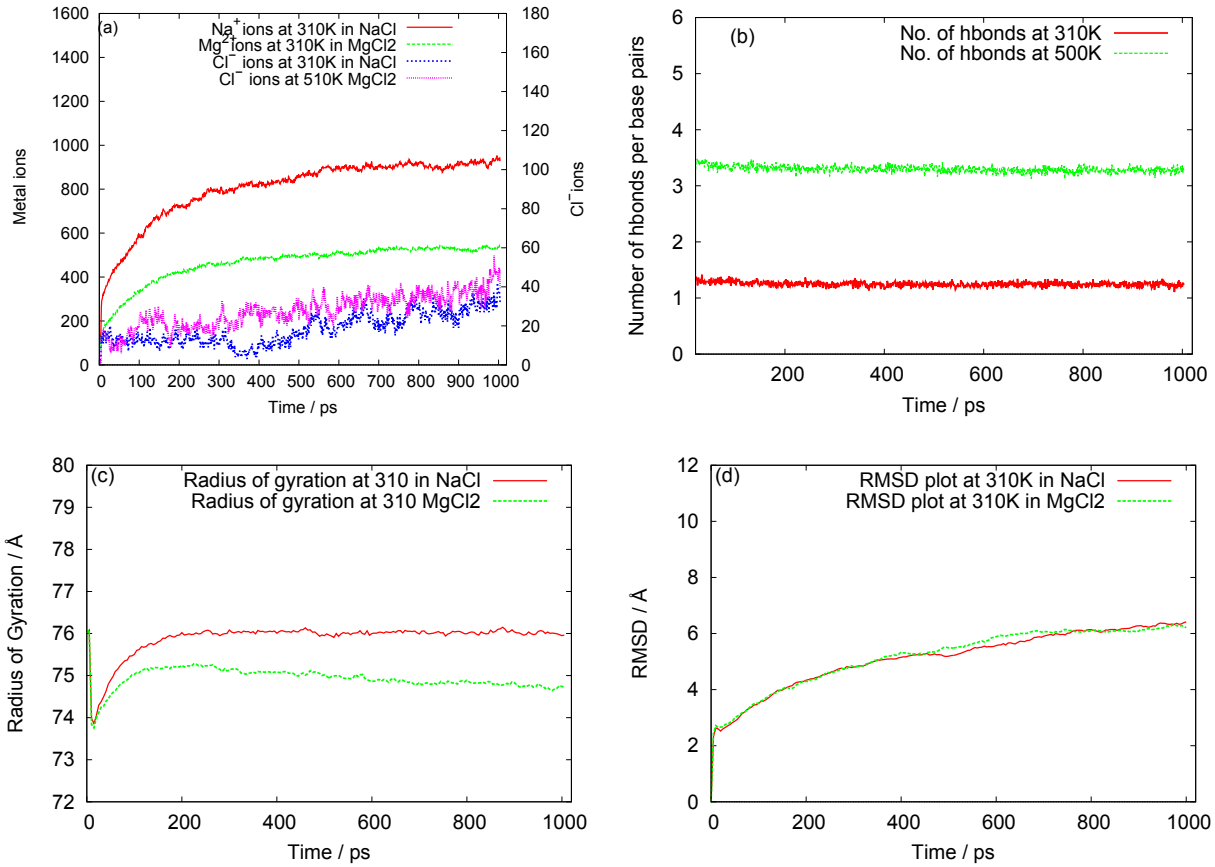


Figure 3: (a) Number of ions with the range of 5 $\AA$ (b) Number of bonds per base pairs (c) Radius of gyration and (d) RMSD of 4 ring RNA nanotube obtained from all atom molecular dynamics simulation.

the RNA nanocluster in MgCl2 solution is found similar to the distribution of the corresponding ions in NaCl solution which is ultimately similar to the characteristics obtained for other various RNA nanoclusters in physiological solution [4, 5]. Furthermore, the results for the radius of gyration, root mean square deviation and the number of basepairs in two different solutions are presented in Figure 3(b),(c) and (d) respectively. From the plots the radius of gyration of the system is increasing at the beginning which later becomes constant for all solutions. The value for the radius of gyration in the MgCl2 solution is higher than that in the NaCl solution. The root mean square deviation is not significantly changing on going from one to another salt solution.

In our earlier studies we have analysed the properties of the RNA nanocluster at different temperatures as well as the different concentration of the physiological solution. In current study the calculation of the properties in different kind of salt solution is done. The temperature and the concentration are taken constant during molecular dynamics simulation.

4 Conclusions

In this paper we have presented four ring RNA nanotube constructed from the RNA building blocks as an example of a coupled problem in studying complex RNA based biological systems. Its behaviour in different metal chloride solutions has been studied. The change in the behaviour of the RNA nanotube in these solutions are found similar to the results obtained in our earlier work those were performed for the RNA nanoclusters of the various size [3, 4, 5]. From our analysis, it is clear that the quality of the results are likely to be improved further by doing the MD simulation for longer period of time those are comparable to the time of the real biological process that occur in the human body. Furthermore, the study of the properties of the RNA nanocluster at the different concentration of $^{25}Mg^{2+}$ions in the solution will give a better understanding about the stability of the RNA nanocluster.

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